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Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHOD FOR PREPARING CELL CULTURES FROM BIOLOGICAL SPECIMENS FOR CHEMOTHERAPEUTIC AND OTHER ASSAYS

(57) Abstract: An improved method for preparing a cell culture is disclosed. The method includes culturing a multicellular tissue explant in the presence of growth medium that is substantially free of enzymes capable of digesting the explant and, subsequently, removing the explant at a predetermined timed.

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METHOD FOR PREPARING CELL CULTURES FROM BIOLOGICAL SPECIMENS FOR CHEMOTHERAPEUTIC AND OTHER ASSAYS

RELATED APPLICATIONS

[0001] This application claims priority, and the benefit of U.S. Serial No. 10/208,480, filed July 30, 2002, the disclosure of which is incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

[0002] The invention relates to methods for the preparation of a cell culture monolayer, and more particularly to methods for the preparation of a tumor cell culture monolayer that substantially comprises tumor cells.

BACKGROUND

[0003] Prior to approval for medical use in the United States, all pharmaceutical agents are subjected to rigorous testing for efficacy and safety. Typically, methods of assessing the efficacy of a pharmaceutical agent include complex studies of pooled patient samples or pooled data, and statistical interpretation of the results. The conclusions that follow such studies are inherently generalized or averaged over the subject patient population. With pharmaceutical agents, however, and particularly with cancer chemotherapeutic agents, the efficacy of an agent in treating an individual patient can vary greatly from the generalized data, often to the detriment of the individual patient. The need has long been recognized for a method of assessing the therapeutic potential of pharmaceutical agents, including but not limited to chemotherapeutic agents, for their specific efficacy in an individual patient.

[0004] Assays exist which expose malignant tissue of various types to pharmaceutically-active agents for the purpose of assessing the best choice for therapeutic administration. For example, in Kruczynski, A., et al., "Evidence of a direct relationship between the increase in the *in vitro* passage number of human non-small-cell lung cancer primocultures and their chemosensitivity," *Anticancer Research*, vol. 13, no. 2, pp. 507-513 (1993), chemosensitivity of non-small-cell lung cancers was investigated in *in vivo* grafts, in *in vitro* primocultures, and in commercially available cancer cell lines. The increase in chemosensitivity was documented and correlated with morphological changes in the cells in question. Often, animal model malignant cells and/or established cell cultures are tested with prospective therapy agents, see for example

selecting said chemotherapeutic agent for treating said patient if said chemotherapeutic agent effects cellular phenotype of said sample of cells in said conducting step and if said cells in said determining step do not comprise a genotypic characteristic associated with resistance to said chemotherapeutic agent.

9. The method of claim 8, wherein said sample of cells in said conducting step comprise malignant cells.

10. The method of claim 8, wherein said sample of cells in said conducting step comprise abnormal proliferating cells.

11. The method of claim 8, wherein said cellular phenotype is cell growth rate.

12. The method of claim 8, wherein said genotypic change is a genetic polymorphism.

13. The method of claim 8, wherein said determining step comprises sequencing a portion of the genome of cells from said patient.

14. The method of claim 8, wherein said determining step comprises comparing said genotype characteristics to a database of genotype characteristics associated with resistance to said chemotherapeutic agent.

15. A method for assessing efficacy of a chemotherapeutic agent on malignant cells in a patient, the method comprising:

exposing malignant cells from a patient to a chemotherapeutic agent;

conducting an assay to determine whether said chemotherapeutic agent effects cellular phenotype of said malignant cells from said patient;

determining whether a sample of cells from said patient comprise a genotypic characteristic associated with resistance to said chemotherapeutic agent; and

assessing efficacy of said chemotherapeutic agent on said malignant cells from said patient based upon results of said conducting and detecting steps.

16. The method of claim 15, further comprising:

selecting said chemotherapeutic agent for treating said patient if said chemotherapeutic agent effects cellular phenotype of said malignant cells in said conducting step and if said cells in said determining step do not comprise a genotypic characteristic associated with resistance to said chemotherapeutic agent.

17. The method of claim 15, wherein said malignant cells are obtained from a tumor specimen from said patient.

18. The method of claim 15, wherein said cells in said determining step are obtained from a blood sample from said patient.

19. The method of claim 15, wherein said cells in said determining step are obtained from a buccal smear from said patient.

20. The method of claim 15, wherein said cellular phenotype is cell growth rate.

21. The method of claim 15, wherein said genotypic change is a genetic polymorphism.

22. The method of claim 15, wherein said determining step comprises sequencing a portion of the genome of cells from said patient.

23. The method of claim 15, wherein said determining step comprises comparing said genotype characteristics to a database of genotype characteristics associated with resistance to said chemotherapeutic agent.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2003/032285

A. CLASSIFICATION OF SUBJECT MATTER

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
Name and mailing address of the ISA/ Facsimile No.	Authorized officer Telephone No.

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:

OCZEK, Jeremy, P.
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High Street Tower
125 High Street
Boston, MA 02110
ETATS-UNIS D'AMERIQUE

Date of mailing *(day/month/year)* 29 April 2004 (29.04.2004)	
Applicant's or agent's file reference PTI-007PC	**IMPORTANT NOTICE**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2003/032285	10 October 2003 (10.10.2003)	10 October 2002 (10.10.2002)

Applicant

PRECISION THERAPEUTICS, INC.

1. Notice is hereby given that the International Bureau has **communicated**, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this notice:

   AU, AZ, BY, CH, CN, CO, DZ, EP, HU, JP, KG, KP, KR, MD, MK, MZ, RU, TM

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:

   AE, AG, AL, AM, AP, AT, BA, BB, BG, BR, BZ, CA, CR, CU, CZ, DE, DK, DM, EA, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, ID, IL, IN, IS, KE, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MG, MN, MW, MX, NI, NO, NZ, OA, OM, PG, PH, PL, PT, RO, SC, SD, SE, SG, SK, SL, SY, TJ, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW

   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-*bis*)).

3. Enclosed with this notice is a copy of the international application as published by the International Bureau on 29 April 2004 (29.04.2004) under No. WO 2004/035833

4. **TIME LIMITS for filing a demand for international preliminary examination and for entry into the national phase**

   The applicable time limit for entering the national phase will, **subject to what is said in the following paragraph, be 30 MONTHS** from the priority date, not only in respect of any elected Office if a demand for international preliminary examination is filed before the expiration of **19 months** from the priority date, but also in respect of any designated Office, in the absence of filing of such demand, where Article 22(1) as modified with effect from 1 April 2002 applies in respect of that designated Office. For further details, see *PCT Gazette* No. 44/2001 of 1 November 2001, pages 19926, 19932 and 19934, as well as the *PCT Newsletter*, October and November 2001 and February 2002 issues.

   In practice, **time limits other than the 30-month time limit** will continue to apply, for various periods of time, in respect of certain designated or elected Offices. For **regular updates on the applicable time limits** (20, 21, 30 or 31 months, or other time limit), Office by Office, refer to the *PCT Gazette*, the *PCT Newsletter* and the *PCT Applicant's Guide*, Volume II, National Chapters, all available from WIPO's Internet site, at http://www.wipo.int/pct/en/index.html.

   For filing a **demand for international preliminary examination**, see the *PCT Applicant's Guide*, Volume I/A, Chapter IX. Only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination (at present, all PCT Contracting States are bound by Chapter II).

   It is the applicant's **sole responsibility** to monitor all these time limits.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Philippe Becamel
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